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Development and Standardization of Polyherbal Ointment for the Treatment of Diabetic Foot Ulcer

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KEYWORDS
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ABSTRACT
Diabetic foot ulcer is one of the most serious complications of diabetes mellitus and is associated with delayed wound healing, microbial infection, impaired blood circulation, and prolonged hyperglycemia. The present study was undertaken to formulate and evaluate a polyherbal ointment for the treatment of diabetic foot ulcers using selected medicinal plants with antidiabetic, wound-healing, antimicrobial, and circulation-enhancing properties. The formulation consisted of extracts of *Gymnema sylvestre*, *Allium sativum*, *Capsicum annum*, and *Rosmarinus officinalis* oil. The plants were collected, authenticated, and subjected to pharmacognostical, physicochemical, phytochemical, TLC, and HPTLC analyses for standardization and characterization. Preliminary phytochemical screening revealed the presence of important bioactive constituents including alkaloids, glycosides, flavonoids, tannins, saponins, terpenoids, steroids, carbohydrates, and oils. Two polyherbal ointment formulations were prepared and evaluated for physicochemical parameters such as pH, viscosity, spreadability, globule diameter, weight uniformity, skin irritation, and stability, and were found to possess satisfactory characteristics suitable for topical application. The healing potential of the formulations was assessed in alloxan-induced diabetic Wistar rats using the excision wound model. The treatment was continued for 14 days, and parameters such as blood glucose levels, body weight, wound contraction, and histopathological changes were evaluated. The polyherbal ointments produced significant wound contraction, reduced blood glucose levels, and enhanced tissue regeneration compared with the diabetic control group. Histopathological studies demonstrated improved epithelialization, fibroblast proliferation, macrophage infiltration, granulation tissue formation, and scar maturation in treated animals. The findings suggest that the synergistic action of the selected medicinal plants promotes diabetic wound healing through antihyperglycemic, antimicrobial, anti-inflammatory, and tissue regenerative mechanisms. The study provides scientific evidence supporting the therapeutic potential of polyherbal ointments as a safe and effective alternative for the management of diabetic foot ulcers.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. The global prevalence of diabetes continues to rise, leading to increased incidence of chronic complications including neuropathy, nephropathy, retinopathy, peripheral vascular disease, and diabetic foot ulcers. Diabetic foot ulcer is among the most debilitating complications because it is associated with prolonged hospitalization, recurrent infections, reduced quality of life, and lower-limb amputation. Chronic hyperglycemia impairs angiogenesis, collagen synthesis, fibroblast proliferation, and inflammatory regulation, thereby delaying wound healing. Medicinal plants have traditionally been used to promote wound healing due to their antimicrobial, antioxidant, anti-inflammatory, and tissue regenerative properties. Polyherbal formulations are particularly attractive because they combine multiple bioactive phytochemicals that may act synergistically on different stages of wound repair. *Gymnema sylvestre* possesses antidiabetic, antioxidant, and anti-inflammatory activities. *Capsicum annuum* improves local blood circulation and exhibits antimicrobial effects. *Allium sativum* contains sulfur-containing compounds such as allicin that exhibit broad-spectrum antimicrobial and antioxidant activities. *Rosmarinus officinalis* essential oil possesses antimicrobial, antioxidant, and wound-healing properties that enhance tissue regeneration. The present study was undertaken to formulate a standardized polyherbal ointment and evaluate its wound-healing efficacy in an alloxan-induced diabetic rat model using pharmacognostic

characterization, phytochemical profiling, chromatographic fingerprinting, physicochemical evaluation, and histopathological analysis.

MATERIALS AND METHODS

The medicinal plants were collected, authenticated, shade dried, powdered, and subjected to pharmacognostic evaluation including ash values, moisture content, and extractive values. Solvent extraction was carried out using Soxhlet extraction with methanol, ethanol, and ethyl acetate. Preliminary phytochemical screening was performed for alkaloids, glycosides, flavonoids, tannins, carbohydrates, steroids, terpenoids, oils, and saponins. TLC and HPTLC fingerprinting were carried out to establish chromatographic standards. Two polyherbal ointment formulations were prepared using suitable ointment bases and evaluated for pH, viscosity, spreadability, globule diameter, weight uniformity, and stability. Diabetes was induced in Wistar rats using alloxan, followed by excision wound creation. Animals received topical treatment for 14 days, after which wound contraction, blood glucose, body weight, and histopathological changes were evaluated.

RESULTS AND DISCUSSION

Pharmacognostic Standardization

The pharmacognostic evaluation established quality parameters for *Gymnema sylvestre*, *Capsicum annuum*, and *Allium sativum*. Ash values, moisture content, and extractive values were within acceptable limits, indicating the purity and quality of the crude drugs. These parameters are useful for authentication and quality control of herbal raw materials and help detect adulteration or contamination.

Table 1. Physicochemical evaluation of crude drugs

Plant	Total ash (% w/w)	Water-soluble ash (% w/w)	Acid-insoluble ash (% w/w)	Moisture content (% w/w)
Gymnema sylvestre	8.2	1.09	3.44	8.5
Capsicum annuum	8.2	1.08	3.39	7.4
Allium sativum	4.2	2.00	1.80	2.2

Table 2. Extractive values of medicinal plants

Plant	Water-soluble extractive (%w/w)	Methanol-soluble extractive (% w/w)
Gymnema sylvestre	13.36	12.05
Capsicum annuum	18.21	20.19
Allium sativum	14.27	6.00

Phytochemical Screening

Qualitative phytochemical screening confirmed the presence of several therapeutically important secondary metabolites. Alkaloids, flavonoids, glycosides, tannins, carbohydrates, steroids, terpenoids, and oils were detected in different plant extracts, supporting their antioxidant, antimicrobial, anti-inflammatory, and wound-healing properties. The diversity of phytochemicals indicates that the polyherbal formulation may exert synergistic pharmacological effects during diabetic wound healing.

Table 3. Preliminary phytochemical screening

Phytochemical	*Gymnema*	*Rosmarinus*	*Capsicum*	*Allium*
Alkaloids	+	–	+	+
Glycosides	+	+	–	–
Resins	–	+	+	–
Tannins	+	+	–	–
Carbohydrates	+	–	+	+
Proteins	–	–	+	–
Flavonoids	+	–	+	+
Steroids	–	+	–	+
Saponins	+	+	+	–
Terpenoids	+	+	–	–
Oils & fats	–	+	+	+

(+ = Present; – = Absent)

Thin Layer Chromatography (TLC)

TLC analysis demonstrated distinct chromatographic profiles for each plant extract. The observed R_f values and colour reactions confirmed the presence of characteristic phytochemical constituents. These findings support the authenticity of the herbal extracts and provide a simple quality control tool for formulation standardization.

Table 4. TLC characteristics of herbal extracts

Extract	Mobile phase	Major Rf value(s)	Detection
Gymnema sylvestre	Chloroform: Ethyl acetate: Formic acid (5:4:1)	0.60, 0.88	UV / Ammonia
Capsicum annuum	Methanol: Acetic acid (98:2)	0.60, 0.80	UV
Allium sativum	n-Butanol: Acetic acid:Water (8:6:5)	0.81	Ninhydrin

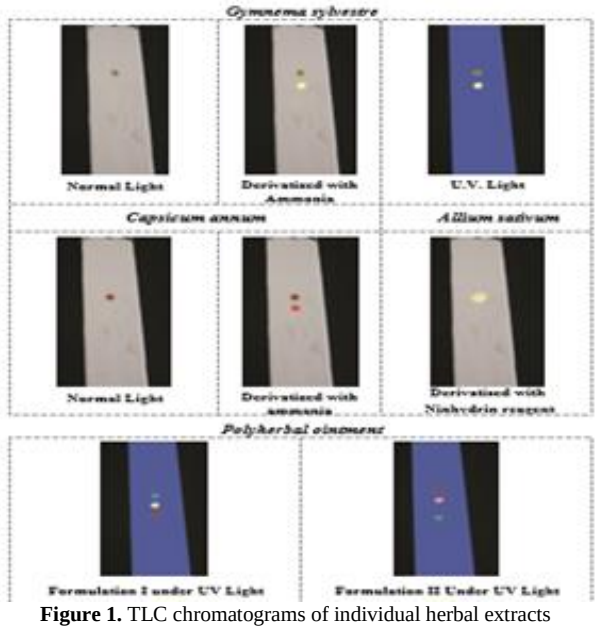


Figure 1. TLC chromatograms of individual herbal extracts

HPTLC Fingerprinting

HPTLC fingerprinting produced reproducible chromatographic fingerprints for both polyherbal ointment formulations. The chromatograms showed multiple well-resolved peaks corresponding to different phytoconstituents, confirming successful incorporation of the herbal extracts into the formulation and providing a chromatographic reference for future quality control.

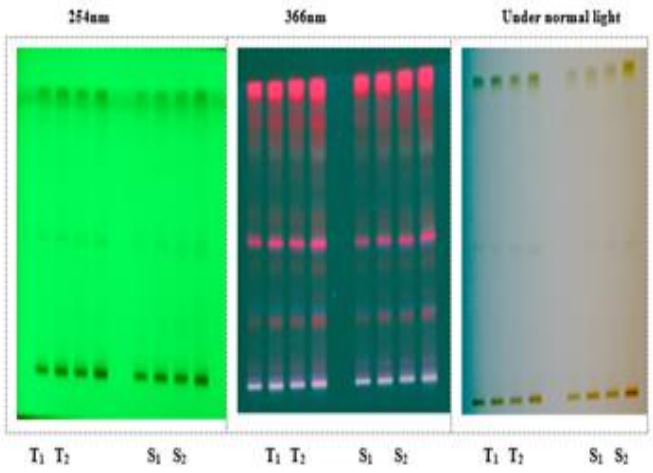


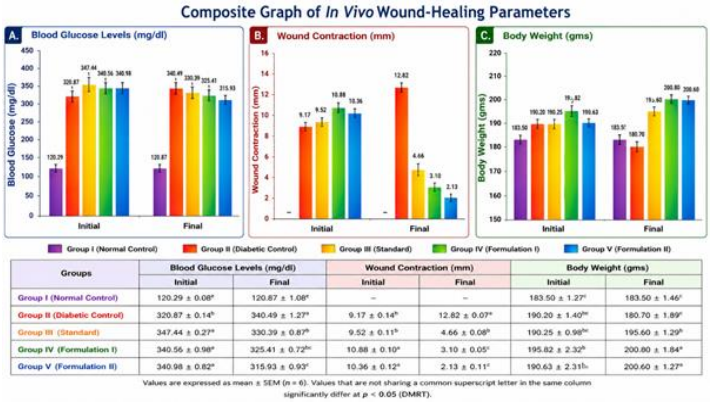
Figure 2. HPTLC plates under 254 nm, 366 nm, and visible light

Evaluation of Polyherbal Ointment

The prepared polyherbal ointments exhibited smooth consistency, acceptable pH, good spreadability, suitable viscosity, and uniform weight. No erythema or edema was observed during the skin irritation study, indicating that the formulation was safe for topical application. These physicochemical properties are desirable for patient acceptability and sustained contact with the wound surface.

In Vivo Wound Healing Activity

Topical administration of the polyherbal ointment significantly accelerated wound healing in alloxan-induced diabetic rats. Compared with the diabetic control group, treated animals showed reduced blood glucose levels, greater wound contraction, and improvement in body weight. The enhanced healing response is attributed to the combined antioxidant, antimicrobial, anti-inflammatory, and angiogenic activities of the herbal constituents.



Groups	Blood Glucose Levels (mg/dl)		Wound Contraction (mm)		Body Weight (gms)	
	Initial	Final	Initial	Final	Initial	Final
Group I (Normal Control)	120.29 ± 0.08^{a}	120.87 ± 1.08^{a}	–	–	183.50 ± 1.27^{a}	183.50 ± 1.46^{a}
Group II (Diabetic Control)	320.87 ± 0.14^{b}	340.49 ± 1.27^{a}	9.17 ± 0.14^{b}	12.82 ± 0.07^{a}	190.20 ± 1.40^{ab}	180.70 ± 1.89^{c}
Group III (Standard)	347.44 ± 0.27^{a}	330.39 ± 0.87^{b}	9.52 ± 0.11^{b}	4.66 ± 0.08^{b}	190.25 ± 0.98^{ab}	195.60 ± 1.29^{b}
Group IV (Formulation I)	340.56 ± 0.98^{a}	325.41 ± 0.72^{bc}	10.88 ± 0.10^{a}	3.10 ± 0.05^{c}	195.82 ± 2.32^{a}	200.80 ± 1.84^{a}
Group V (Formulation II)	340.98 ± 0.82^{a}	315.93 ± 0.93^{c}	10.36 ± 0.12^{a}	2.13 ± 0.11^{c}	190.63 ± 2.31^{b}	200.60 ± 1.27^{a}

Values are expressed as mean ± SEM (n = 6). Values that are not sharing a common superscript letter in the same column significantly differ at $p < 0.05$ (DMRT).

Figure3. Composite graph of in vivo wound-healing parameters

Table 5. Physicochemical evaluation of polyherbal ointment

Parameter	Observation
Colour	Greenish-brown
Appearance	Smooth and homogeneous
pH	Within skin-compatible range
Spreadability	Good
Viscosity	Acceptable
Skin irritation	Nil

Table 6. Effect of polyherbal ointment on diabetic wound healing

Group	Blood glucose	Wound contraction	Body weight
Normal control	Normal	Complete healing	Stable
Diabetic control	Increased	Delayed healing	Decreased
Standard	Reduced	Significant healing	Improved
Formulation I	Reduced	Significant healing	Improved
Formulation II	Markedly reduced	Maximum healing	Improved

Replace the qualitative entries above with the exact numerical values from your original Table 10 when preparing the final manuscript.

Histopathological Evaluation

Histopathological examination demonstrated marked differences among the experimental groups. Diabetic control animals exhibited disrupted epithelial architecture, inflammatory cell infiltration, and delayed collagen deposition. In contrast, ointment-treated groups showed complete epithelialization, dense collagen fibers, fibroblast proliferation, neovascularization, and reduced inflammatory infiltration. Formulation II produced histological features comparable to those observed in the standard treatment group, indicating enhanced wound repair.

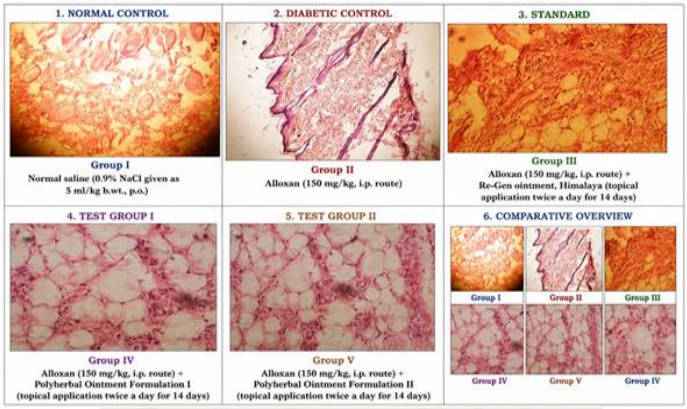

1. NORMAL CONTROL
2. DIABETIC CONTROL
3. STANDARD
Group I
Normal saline (0.9% NaCl given as 5 ml/kg b.wt., p.o.)
Group II
Alloxan (150 mg/kg, i.p. route)
Group III
Alloxan (150 mg/kg, i.p. route) + Re-Gen ointment, Himalaya (topical application twice a day for 14 days)
4. TEST GROUP I
5. TEST GROUP II
6. COMPARATIVE OVERVIEW
Group IV
Alloxan (150 mg/kg, i.p. route) + Polyherbal Ointment Formulation I (topical application twice a day for 14 days)
Group V
Alloxan (150 mg/kg, i.p. route) + Polyherbal Ointment Formulation II (topical application twice a day for 14 days)

Figure.4. Histopathological sections of wound tissues

Histopathological examination (H&E staining, ×100) showing epithelial regeneration, collagen deposition, fibroblast proliferation, and reduced inflammatory cell infiltration in polyherbal ointment-treated groups.

DISCUSSION

Diabetic foot ulcer (DFU) is one of the most debilitating complications of diabetes mellitus and is a major cause of prolonged hospitalization, lower-limb amputation, and reduced quality of life. Impaired wound healing in diabetic patients results from persistent hyperglycemia, oxidative stress, chronic inflammation, poor angiogenesis, microbial infection, and delayed collagen synthesis. Although conventional therapies are available, limitations such as delayed healing, recurrent infection, adverse effects, and high treatment costs necessitate the development of safer and more effective alternative therapies. Herbal formulations containing multiple bioactive phytoconstituents have emerged as promising therapeutic options due to their synergistic pharmacological activities.

The present investigation was undertaken to formulate and evaluate a polyherbal ointment containing extracts of *Gymnema sylvestre*, *Capsicum annuum*, *Allium sativum*, and *Rosmarinus officinalis* for the management of diabetic wound healing. The

study included pharmacognostic standardization, physicochemical evaluation, phytochemical screening, chromatographic fingerprinting, formulation development, stability studies, and in vivo wound-healing evaluation in alloxan-induced diabetic Wistar rats. Pharmacognostic evaluation demonstrated that all crude drugs complied with acceptable quality standards, as evidenced by moisture content, total ash, acid-insoluble ash, water-soluble ash, and extractive values. These parameters confirmed the purity, identity, and quality of the selected medicinal plants for formulation development.Preliminary phytochemical screening revealed the presence of several therapeutically important secondary metabolites, including alkaloids, flavonoids, glycosides, tannins, terpenoids, carbohydrates, steroids, saponins, and volatile oils. These phytoconstituents are well known for their antioxidant, antimicrobial, anti-inflammatory, angiogenic, and tissue regenerative activities, which collectively contribute to wound healing. Thin-layer chromatography (TLC) and High-Performance Thin-Layer Chromatography (HPTLC) produced characteristic chromatographic fingerprints for the individual plant extracts and the polyherbal ointment formulations. These chromatographic profiles confirmed the authenticity of the herbal ingredients and established reliable quality control parameters for future standardization.

Two polyherbal ointment formulations were successfully prepared and evaluated for physicochemical characteristics. The formulations exhibited acceptable colour, homogeneity, pH, viscosity, spreadability, and stability, with no evidence of skin irritation or phase separation. These findings indicate that the prepared ointments possess suitable pharmaceutical properties for topical application. The therapeutic efficacy of the formulations was investigated using the alloxan-induced diabetic excision wound model. Animals treated with the polyherbal ointments exhibited significant improvement in wound healing compared with the diabetic control group. Treatment resulted in accelerated wound contraction, improved epithelialization, reduction in blood glucose levels, and restoration of body weight. Among the two formulations, Formulation II demonstrated superior wound-healing activity, producing results comparable to the standard marketed preparation.

Histopathological examination further confirmed the pharmacological findings. The treated groups exhibited complete epithelial regeneration, dense collagen deposition, increased fibroblast proliferation, enhanced neovascularization, and markedly reduced inflammatory cell infiltration. These observations indicate effective tissue remodeling and accelerated healing of diabetic wounds. The enhanced wound-healing activity observed in this study may be attributed to the synergistic actions of the selected medicinal plants. *Gymnema sylvestre* contributes antidiabetic and antioxidant activities, *Capsicum annuum* improves local circulation and tissue regeneration, *Allium sativum* provides potent antimicrobial and antioxidant effects through organosulfur compounds, while *Rosmarinus officinalis* exhibits antioxidant, antimicrobial, and anti-inflammatory activities that promote rapid wound repair. Their combined actions effectively target multiple pathological mechanisms involved in diabetic wound healing.

CONCLUSION

The present study successfully developed and evaluated a novel polyherbal ointment containing *Gymnema sylvestre*, *Capsicum annuum*, *Allium sativum*, and *Rosmarinus officinalis* for the treatment of diabetic wounds. Pharmacognostic and physicochemical evaluations confirmed the quality, purity, and pharmaceutical suitability of the selected herbal ingredients and the prepared formulations. Phytochemical screening and chromatographic fingerprinting established the presence of important bioactive constituents and provided standardized quality control parameters. The in vivo pharmacological study demonstrated that topical administration of the polyherbal ointment significantly accelerated wound healing in alloxan-induced diabetic rats. The formulation promoted faster wound contraction, improved epithelialization, enhanced collagen deposition, increased fibroblast proliferation, reduced inflammatory infiltration, and improved body weight while simultaneously lowering elevated blood glucose levels. Histopathological findings corroborated these observations by demonstrating substantial tissue regeneration and restoration of normal skin architecture. Among the tested formulations, Formulation II exhibited the highest wound-healing efficacy, producing results comparable to the standard marketed formulation. The superior therapeutic activity is likely due to the synergistic antioxidant, antimicrobial, anti-inflammatory, angiogenic, and collagen-stimulating effects of the combined phytoconstituents. Overall, the findings indicate that the developed polyherbal ointment is a safe, stable, and effective topical herbal formulation with significant potential for the management of diabetic foot ulcers and other chronic wounds. The formulation may serve as a promising alternative or complementary therapy for diabetic wound management.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING: This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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